Acta Biomaterialia 10 (2014) 4996–5004

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

# Injectable carboxymethylcellulose hydrogels for soft tissue filler applications

Devika M. Varma [a], Gittel T. Gold [a], Peter J. Taub [b], Steven B. Nicoll [a,*]

[a] Department of Biomedical Engineering, The City College of New York, New York, NY 10031, USA
[b] Division of Plastic and Reconstructive Surgery, The Mount Sinai Medical Center, New York, NY 10029, USA

ARTICLE INFO

*Article history:*
Received 10 January 2014
Received in revised form 1 August 2014
Accepted 12 August 2014
Available online 23 August 2014

*Keywords:*
Soft tissue augmentation
Carboxymethylcellulose
Hydrogel
Gelation
Mechanical properties

ABSTRACT

Disease, trauma and aging all lead to deficits in soft tissue. As a result, there is a need to develop materials that safely and effectively restore areas of deficiency. While autogenous fat is the current gold standard, hyaluronic acid (HA) fillers are commonly used. However, the animal and bacterial origin of HA-based materials can induce adverse reactions in patients. With the aim of developing a safer and more affordable alternative, this study characterized the properties of a plant-derived, injectable carboxymethylcellulose (CMC) soft tissue filler. Specifically, methacrylated CMC was synthesized and crosslinked to form stable hydrogels at varying macromer concentrations (2–4% w/v) using an ammonium persulfate and ascorbic acid redox initiation system. The equilibrium Young's modulus was shown to vary with macromer concentration (ranging from ~2 to 9.25 kPa), comparable to values of native soft tissue and current surgical fillers. The swelling properties were similarly affected by macromer concentration, with 4% gels exhibiting the lowest swelling ratio and mesh size, and highest crosslinking density. Rheological analysis was performed to determine gelation onset and completion, and was measured to be within the ISO standard for injectable materials. In addition, hydrolytic degradation of these gels was sensitive to macromer concentration, while selective removal using enzymatic treatment was also demonstrated. Moreover, favorable cytocompatibility of the CMC hydrogels was exhibited by co-culture with human dermal fibroblasts. Taken together, these findings demonstrate the tunability of redox-crosslinked CMC hydrogels by varying fabrication parameters, making them a versatile platform for soft tissue filler applications.



## 1. Introduction

Soft tissue fillers have been used for decades for reconstructive and aesthetic procedures. Aging, trauma and disease result in the loss of dermal collagen and fat, requiring intervention. Several natural and synthetic fillers have been used clinically for tissue augmentation and to restore tissue structure and function [1,2]. Autogenous fat is the gold standard [3,4] for soft tissue replacement due to its completely natural composition. Adipose tissue may be harvested from a remote donor site in the same patient and reinjected into an area of deficiency. The benefits of autogenous fat include its biocompatibility, relative low cost and permanence, while the inherent disadvantages are potential donor site morbidity, unpredictable survival due to dependence on a vascular bed [5] and limited supply if larger volumes are required [6,7].

Alternatives to fat grafts include a variety of natural and synthetic biomaterials that can be broadly categorized into either permanent or temporary fillers [8]. Recently, semipermanent or temporary fillers such as hyaluronic acid (HA) and collagen have been widely used due to their natural origin; however, the source (i.e. animal or bacterial) predisposes them to foreign body reactions [9]. Additionally, both HA and collagen are major components of natural tissue, making them susceptible to enzymatic degradation and significantly lowering their lifespan to a few weeks or months [9]. An ideal temporary filler material should be injectable, biocompatible and mechanically stable over a long term but also resorbable. In particular, patients suffering from HIV-induced lipoatrophy prefer fillers that do not remain in situ permanently, as the gradual redeposition of facial fat during the recovery process may lead to an absurd overcorrection to the earlier lipoatrophied face [10].

Carboxymethylcellulose (CMC) is an FDA-approved, water-soluble, cellulose-derived polysaccharide that is available in high-purity forms and has found several biomedical applications due to its biocompatibility and low cost [11]. Given its plant-based origin, CMC is less likely to elicit an immune response, which is a key advantage over other animal-derived natural fillers, such as collagen and HA [12–14]. Furthermore, the absence of the cellulose-digesting

* Corresponding author. Tel.: +1 212 650 6237; fax: +1 212 650 6727.
*E-mail address:* snicoll@ccny.cuny.edu (S.B. Nicoll).

http://dx.doi.org/10.1016/j.actbio.2014.08.013
1742-7061/

enzyme, cellulase, in humans affords suitable mechanical stability of CMC in vivo, in comparison to other natural biomaterial fillers that are susceptible to enzymatic activity [15].

Several commercially available dermal fillers such as Laresse, Radiesse and Sculptra [16] incorporate CMC with other materials (i.e. poly(ethylene oxide), hydroxyapatite and poly(lactic acid)). Most of these fillers utilize uncrosslinked formulations of CMC [17], which can potentially reduce their mechanical stability and retention time in vivo. Furthermore, the presence of synthetic components in these fillers can make selective removal following adverse reactions, migration or malposition very challenging, requiring invasive surgical procedures [8,18,19]. Recently, UV-light-induced free radical polymerization was employed to fabricate covalently crosslinked CMC hydrogels [20,21]. However, the dependence on UV light limits the application of such gels as injectable filler materials due to poor light penetration through tissues. Reduction–oxidation (redox) initiation systems may serve as alternatives to such UV-based polymerization techniques. For example, the redox initiators ammonium persulfate (APS) and ascorbic acid (AA) have been used previously to engineer synthetic hydrogel scaffolds, but not for naturally derived materials, including CMC [22,23]. In addition, the development of a hydrogel that is injected in an uncrosslinked form and cures in situ would present distinct advantages in terms of handling and administration, compared to existing commercial fillers that are crosslinked prior to injection. Therefore, the objective of this study was to develop redox-polymerized CMC hydrogels and characterize their material properties in vitro in order to assess their potential as injectable soft tissue fillers.

## 2. Materials and methods

### 2.1. Macromer synthesis

Methacrylation of 250 kDa CMC (Sigma-Aldrich) was performed based on a previous protocol [24,25]. Briefly, a 1% (w/v) solution of 250 kDa CMC was reacted with a 20-fold excess of methacrylic anhydride (Sigma-Aldrich) in sterile water at 4 °C and a pH of 8 over 24 h (Fig. 1). To remove excess, unreacted methacrylic anhydride, the CMC solution was dialyzed against sterile water for over 96 h. Purified CMC solution was lyophilized to obtain a solid product which was stored at −20 °C. To confirm the degree of methacrylation, the modified polymer underwent acid hydrolysis and the product was analyzed with $^1$H nuclear magnetic resonance spectroscopy (NMR) at 300 MHz (Varian Mercury 300, Agilent Technologies) [20,26]. Molar methacrylation percentage was calculated by integrating methacrylate proton peaks (methylene, δ = 6.0 and 5.6 ppm and the methyl peak, δ = 1.8 ppm) relative to carbohydrate protons.

### 2.2. Hydrogel preparation

Methacrylated CMC was dissolved in Dulbecco's phosphate-buffered saline (DPBS) (Invitrogen) at 4 °C to obtain a homogeneous solution which was combined with APS (10 mM) (Sigma-Aldrich) and AA (10 mM) (Sigma-Aldrich) to obtain solutions at final macromer concentrations of 2, 3 and 4% (w/v) (Fig. 1). These concentrations were chosen to satisfy specific sample preparation and handling criteria for an injectable material (i.e. ease of resuspension). Polymer-initiator solutions were poured into custom-made casting devices and allowed to set for 15 min at room temperature to form cylindrical gels 5 mm in diameter × 2 mm thick (unless stated otherwise).

### 2.3. Swelling ratio and related physical properties

Swelling of CMC hydrogels and their associated physical properties were analyzed by calculating the equilibrium weight swelling ratio, $Q_w$, at day 1 ($n = 4$) for the 5 mm diameter × 2 mm thick hydrogels at macromer concentrations of 2, 3 and 4% (w/v). Constructs were weighed to determine the wet weight ($W_s$) after an overnight incubation at 37 °C in DPBS, lyophilized overnight, and then reweighed to measure the dry weight ($W_d$). $Q_w$ was calculated using the following equation:

$$Q_w = \frac{W_s}{W_d}. \tag{1}$$

The crosslinking density ($v_e$) and mesh size ($\xi$) were calculated based on the Flory–Rehner model as was previously described [27–30]. Briefly, the volumetric swelling ratio ($Q_v$) was determined from the mass swelling ratio, $Q_w$ [28,31]:

$$Q_v = 1 + \left(\frac{\rho_p}{\rho_s}(Q_w - 1)\right), \tag{2}$$

where $\rho_p$ is the density of the dry polymer (0.52 g cm$^{-3}$) [32] and $\rho_s$ is the density of the solvent (water = 1 g cm$^{-3}$). A simplified version of the Flory–Rehner equation [27,28] was used to calculate the average molecular weight between crosslinks ($\bar{M}_c$) from the following equation:

$$Q_v^{5/3} \cong \frac{\bar{v}\bar{M}_c}{V_l}\left(\frac{1}{2} - \chi\right), \tag{3}$$

where $\bar{v}$ is the specific volume of the dry polymer, $V_l$ is the molar volume of the solvent (water = 18 mol cm$^{-3}$), and $\chi$ is the Flory polymer–solvent interaction parameter (0.473). The value for $\chi$ was based on the assumption that $\chi$ for CMC is comparable to that for other polysaccharides (i.e. HA and dextran) due to similar chemical structures [28,29]. In addition, previous work on other

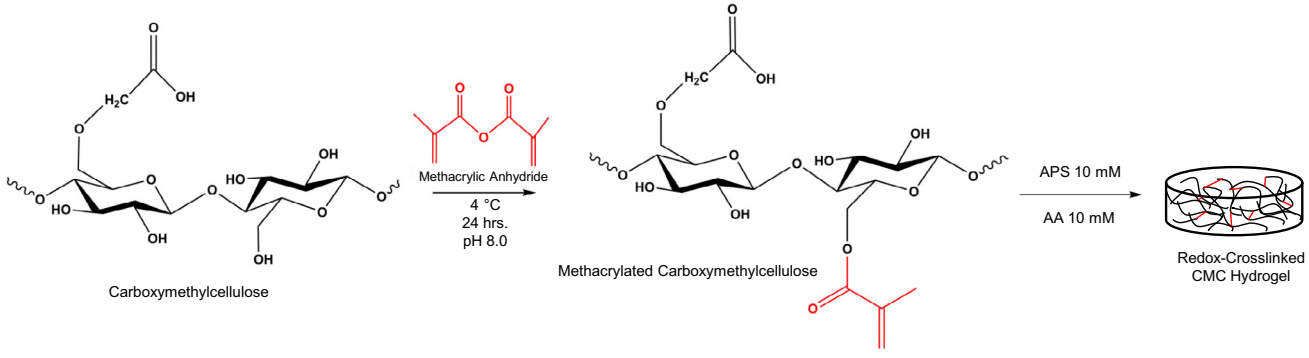


**Fig. 1.** Schematic of methacrylated CMC synthesis and CMC hydrogel fabrication using redox initiators.